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Diethyldithiocarbamate increases distribution of cadmium to brain but prevents cadmium-induced neurotoxicity

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Dithiocarbamates exhibit potent metal-binding properties which have been exploited in a variety of applications, one of which is chelation therapy for heavy metal toxicity. Such therapy, however, promotes the accumulation of metals in the brain, a side effect which may result in neurotoxicity. To examine this possibility we used morphological and biochemical indices to assess the effects of diethyldithiocarbamate (DDC) on cadmium-induced neurotoxicity in the newborn rat. Co-administration of DDC prevented the neurotoxic effects of cadmium while causing a persistent increase in the distribution of cadmium to brain.

Substituted dithiocarbamates are used commercially for their fungicidal, herbicidal and pesticidal properties[11]. They also find application in the plastics and rubber industries as accelerators and catalysts[11]. Dithiocarbamates are potent chelators of polyvalent metals[11], a property that has been exploited for reducing or preventing lethality in experimental animals[8,10] and humans[17] poisoned with heavy metals. Diethyldithiocarbamate (DDC), a prototype dithiocarbamate, also has proven effective in the prevention of the nephrotoxic side effects of anti-tumor[3] and anti-trypanosome[19] therapy with *cis*-platinum, findings which have led to the promotion of DDC as a clinical rescue agent[3]. Treatment of heavy metal intoxication with DDC, while reducing mortality, generally does not promote elimination of the metal from the organism, but instead, effects a redistribution to other organs or tissues[5,10,16]. For example, the brain, a compartment not readily accessible to inorganic metals, accumulates thallium, lead, copper, nickel, mercury, zinc and cadmium following chelation therapy with DDC[1,4,5,10,16]. This effect, which has been attributed to the formation of lipophilic complexes that pass the blood–brain barrier[5,16], is of concern because exposure of the central nervous system (CNS) to heavy metals often is associated with irreversible brain damage[7]. For this reason several investigators[1,4,16] have raised the possibility that chelation of heavy metals with dithiocarbamates may result in neurotoxicity.

In this study we chose the cadmium-intoxicated newborn rat as a model for investigating the effects of DDC on heavy metal-induced neurotoxicity. Acute administration of cadmium to the neonatal rat causes cytopathological changes in the CNS; resistance of the adult is attributed to the failure of cadmium to penetrate the blood–brain barrier[18]. If DDC potentiates the neurotoxic effects of heavy metals as predicted, then we reasoned that co-administration of cadmium and DDC to the developing rat should potentiate cadmium-induced neurotoxicity. To assess the neurotoxic effects of cadmium we used 3 different criteria: (1) brain weight, (2) brain histology, and (3) radioimmunoassays of the neuron-specific phosphoprotein, synapsin I[13] and the astrocyte-specific protein, glial fibrillary acidic protein (GFAP)[2]. Cadmium (2.75–3.75 mg/kg, s.c.) alone or in combination with DDC (100 mg/kg, i.p.) was administered to Long–

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Evans rats on postnatal day (PND) 5. Saline (0.9%) served as the vehicle control. Prior to dosing, pups were reassigned to litters such that each dam received 4 male and 4 female pups and no more than one male and one female of her own offspring. All measurements were obtained on PND 22.

In previous studies we found that weights of whole brain, or of regions of brain that mature postnatally (e.g. hippocampus or cerebellum), predict the underlying histological, biochemical or functional alterations resulting from early postnatal exposure to known neurotoxicants[12,15]. Data for brain and corresponding body weights obtained in the present study are presented in Table I. Cadmium administration alone (2.75–3.75 mg/kg) caused large dose-related decreases in weights of whole brain and cerebellum. DDC did not affect either whole brain or cerebellar weights and prevented or attenuated the cadmium-induced decreases. Cadmium also caused a reduction in body weight that was not effectively blocked by co-administration of DDC.

Photomicrographs of sagittal sections of whole brain are shown in Fig. 1. In comparison to saline-treated rats (Fig. 1A), the effects of DDC alone (Fig. 1B) and of 2.75 mg/kg of cadmium alone (Fig. 1C) were unremarkable. At a dose of 3.0 mg/kg, however, cadmium caused necrosis of the neostriatum and corpus callosum (Fig. 1D), an effect that was blocked by co-administration of DDC (Fig. 1F). At dosages above 3.5 mg/kg, cadmium caused large cystic cavities in the cerebrum with fatalities of 60–70% by PND 22 (photomicrographs and data not shown). This steep dose–effect function for the neuropathological effects of cadmium is not unlike that of other heavy metals, i.e. the no-effect level for a given toxicity endpoint approaches the dosage necessary for irreversible pathology and ensuing fatalities.

Previously we proposed[14] and subsequently demonstrated[12,15] that the nervous system-specific proteins, synapsin I and GFAP, can be used as sensitive biochemical indicators of neurotoxicity. We applied this approach to neurotoxicity assessment in the present investigation by measuring the amounts of synapsin I and GFAP in neostriatum, the primary site of cadmium-induced damage. Radioimmunoassays of synapsin I and GFAP were performed as previously described[6,15] and the results are shown in Table II. Following the administration of 2.75 mg/kg of cadmium, the dosage that did not result in obvious cytopathology, the amount of synapsin I per striatum (total) was decreased 39% whereas the amount of GFAP per striatum was increased 29%. When expressed per milligram of striatal protein (concentration), synapsin I was decreased 19% and GFAP was increased 80%. These effects, which were indicative of cadmium-induced loss of neurons and reactive gliosis, were blocked by concomitant administration of DDC, which itself was without effect.

All indices of neurotoxicity indicated that DDC prevented, rather than potentiated, cadmium-induced neurotoxicity. To determine the relationship between this protective effect of DDC and cadmium

TABLE I

Effect of cadmium administered alone or in combination with DDC on body, brain and cerebellum weight (g) at postnatal day 22

Each value represents the mean ± (S.E.M.) for at least 4 observations. Cadmium, at dosages of 3.0 mg/kg and above caused fatalities that ranged from 19% (3.0 mg/kg) to 69% (3.75 mg/kg) of the subjects by PND 22.

Dose of Cd^{2+} (mg/kg)	*Dose of DDC (mg/kg)*	*Body wt. (g)*	*Brain wt. (g)*	*Cerebellum wt. (g)*
0.0	0.0	51.9 (1.4)	1.48 (0.02)	0.205 (0.005)
0.0	100	53.7 (1.6)	1.48 (0.02)	0.206 (0.004)
2.75	0.0	53.1 (1.7)	1.36 (0.04)*	0.190 (0.005)*
2.75	100	50.6 (1.7)	1.41 (0.02)	0.192 (0.004)*
3.00	0.0	45.2 (3.2)*	1.13 (0.05)*	0.154 (0.007)*
3.00	100	48.7 (0.7)*	1.41 (0.02)†	0.189 (0.004)*†
3.50	0.0	38.8 (7.8)*	1.07 (0.09)*	0.147 (0.010)*
3.50	100	45.2 (4.9)*	1.37 (0.04)*†	0.190 (0.006)*†
3.75	0.0	39.1 (8.3)*	1.09 (0.07)*	0.146 (0.001)*
3.75	100	40.3 (2.2)*	1.31 (0.06)*†	0.173 (0.012)*†

* Significantly less than control (saline, saline), $P < 0.05$.

† Significantly greater than the group given the same dose of cadmium but in the absence of DDC, $P < 0.05$.

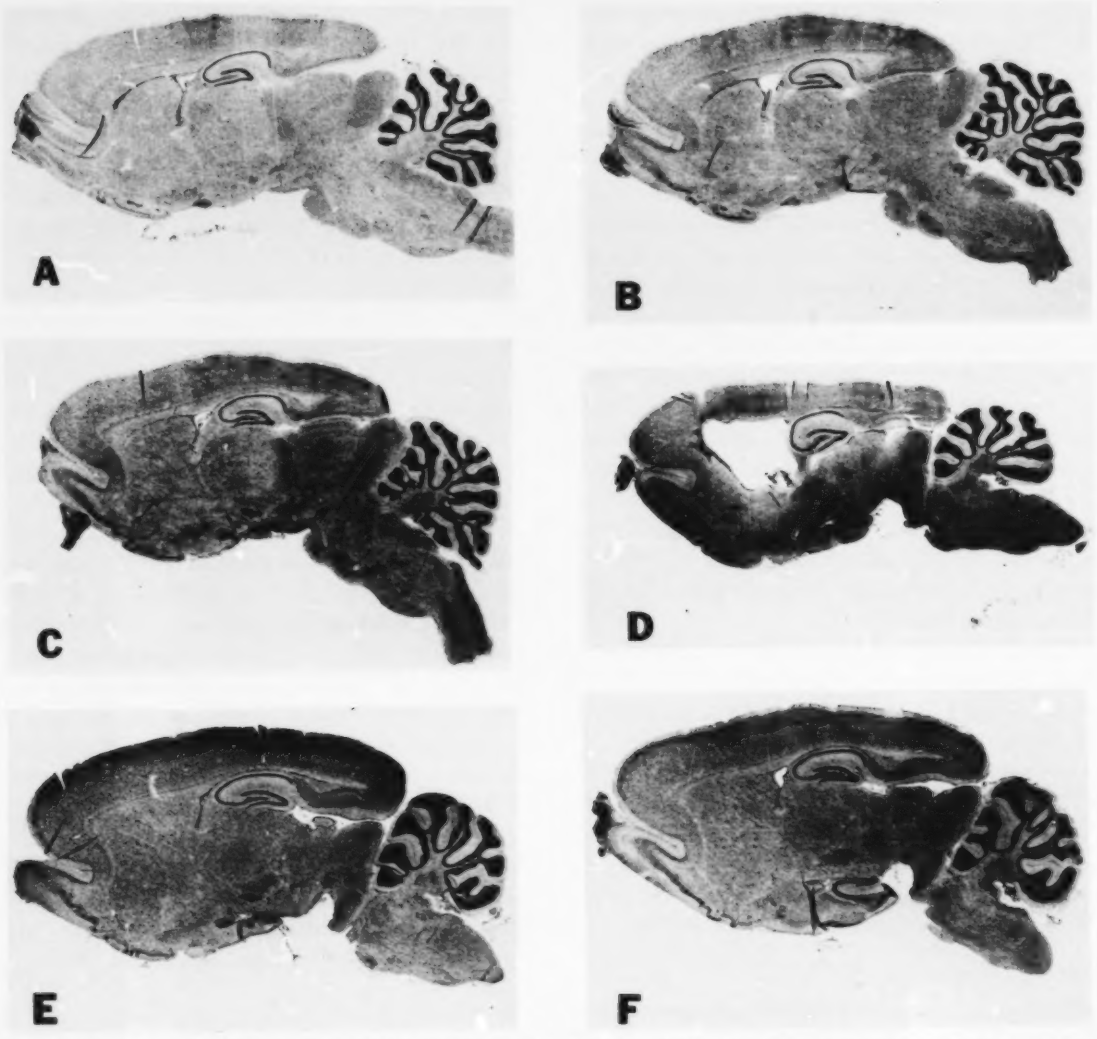

Fig. 1. Morphology of whole brain on PND 22 after administration of cadmium, in the absence and presence of DDC, on PND 5. Sagittal sections of brains (perfused with 0.9% saline followed by buffered 10% Formalin) were prepared, mounted and stained with cresyl violet. A, saline–saline; B, DDC–saline; C, saline–Cd^{2+} (2.75 mg/kg); D, saline-Cd^{2+} (3.0 mg/kg); E, DDC–Cd^{2+} (2.75 mg/kg); and F, DDC–Cd^{2+} (3.0 mg/kg).

distribution, we analyzed cadmium distribution to brain following its administration in the absence and presence of DDC (Table III). Radioisotopic cadmium ($^{109}Cd^{2+}$, carrier-free; New England Nuclear, Boston, MA) was added to a solution of cadmium chloride and administered (s.c.) at a concentration of 40 μCi/kg in the absence and presence of DDC (100 mg/kg, i.p.). Cadmium was measured by scintillation spectrometry. Twenty-four hours after exposure to a dose of 2.75 mg/kg, cadmium distribution to brain represented only 0.32% of the total body burden, an amount that apparently was sufficient to result in neurotoxicity (Table II). Co-administration of DDC increased the total content and concentration of cadmium in brain by 60% and 55%, respectively. By PND 22 values for both content and concentration of cadmium were increased two-fold in the DDC-treated group as compared to the group that received cad-

TABLE II

Effect of cadmium (2.75 mg/kg) administered alone or in combination with DDC (100 mg/kg) on striatal synapsin I and GFAP at postnatal day 22

Each value represents the mean ± (S.E.M.) for at least 14 independent observations. For assays of synapsin I and GFAP, standard curves were constructed from dilutions of a single saline–saline sample. By comparing the immunoreactivity of the test sample with that of the sample used to construct the standard curve, the relative specific activity (RSA) of the sample was obtained; the RSA of an individual sample was multiplied by a factor in order to set the mean RSA of the saline–saline group equal to 1.00 (100%). Data are presented on both a total and concentration basis, i.e. RSA per striatum and RSA per milligram striatal protein. Synapsin I and corresponding antisera were the generous gifts of Drs. J. Chan, M.D. Browning, E. Perdahl and P. Greengard, Rockefeller University. Anti-GFAP was obtained from Dako, Santa Barbara, CA.

Treatment	*Relative specific activity (RSA)*			
	Synapsin I		*GFAP*	
	Total	*Concentration*	*Total*	*Concentration*
Saline–saline	1.00 (0.05)	1.00 (0.05)	1.00 (0.11)	1.00 (0.09)
DDC–saline	1. (0.05)	0.97 (0.04)	0.98 (0.08)	0.91 (0.06)
Saline–cadmium	0.61 (0.06)*	0.81 (0.04)*	1.29 (0.20)	1.80 (0.36)*
DDC–cadmium	0.93 (0.05)	0.93 (0.03)	0.92 (0.09)	0.97 (0.08)

* Significantly different from saline–saline, $P < 0.01$.

mium alone. The decrease in brain cadmium concentration that occurred between PND 6 and PND 22, both in the absence and presence of DDC, could be attributed to the increase in brain weight that occurred over the same period. These data indicate that cadmium accumulates in brain following exposure during the early postnatal period and that concomitant administration of DDC will enhance deposition of cadmium in brain.

TABLE III

Effect of DDC on distribution of cadmium (2.75 mg/kg) to brain

Each value represents the mean ± (S.E.M.) for 16 independent observations. The mean total body burden of cadmium on PND 6 was 38.90 μg.

	μg Cd/brain	*μg Cd/g wet wt.*
Postnatal day 6		
Saline–cadmium	0.124 (0.005)	0.202 (0.007)
DDC–cadmium	0.199 (0.014)*	0.314 (0.021)*
Postnatal day 22		
Saline–cadmium	0.145 (0.003)	0.098 (0.003)
DDC–cadmium	0.291 (0.017)*	0.199 (0.011)*

* Significantly different from cadmium–saline, $P < 0.01$.

In summary, we have demonstrated that co-administration of DDC prevents the morphological and biochemical manifestations of cadmium-induced neurotoxicity while causing a persistent increase in cadmium retention in the brain. These data support the theory that, in vivo, DDC forms a stable lipophilic complex with cadmium and perhaps other metals. In brain, as in other organs, this chelate appears to have little toxicological activity. We do not, however, interpret these findings as an endorsement for the continued or future therapeutic use of DDC as an antidote or rescue agent for heavy metal intoxication. Instead, we suggest that the search for alternatives to DDC therapy[8] should continue, since the protection afforded by DDC chelates may mask the potential for metal-induced neurotoxicity as a consequence of the eventual breakdown[9] or metabolism of the complex.

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